

U.S. Patent Application No. 09/880,654  
 Response to September 20, 2004 Office Action  
 February 22, 2005

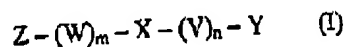
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Claim Listing

1. (previously presented) A method for determining the activity of a protease, said method comprising

- a) incubating a mixture of said protease and a substrate capable of being bound to an anchor, said substrate having a fluorescent radical attached thereto;
- b) binding the substrate to the anchor;
- c) measuring the fluorescence polarization of the mixture; and
- d) correlating measured fluorescence polarization to protease activity.

2. (original) The method of claim 1 wherein the substrate is selected from the compounds of Formula I



wherein X is an amino acid sequence sufficient for substrate recognition by a protease; wherein V and W are independently selected from aminoalkylcarboxylic acids; wherein m and n are numbers independently selected from 0 and 1; and wherein one of Y and Z is a fluorescent radical and the other is a binding radical.

3. (previously presented) The method of claim 2 wherein X is a peptide containing six to sixteen amino acids, inclusive; and wherein V and W are independently selected from the group consisting of glycine, 4-aminobutyric acid, 5-aminopentanoic acid, 6-aminocaproic acid and 7-aminohexanoic acid.

4. (previously presented) The method of claim 3 wherein the anchor is selected from the group consisting of a biotin selective protein, a solid support, and an antibody; wherein the binding radical is selected from the group consisting of biotin, digoxigenin and radicals capable of binding to a solid support; and wherein the fluorescent radical is selected from the group consisting of derivatives of fluorescein, rhodamine, coumarin, eosin, pyrene, quinoline, 5-dimethylamino-naphthalene-1-sulfonyl, dinitrophenyl, benzimidazole, 4-(4-dimethylamino phenylazo)benzoic acid, 5-[(2-aminoethyl)amino]naphthalene-1-sulfonic acid, cascade blue, Texas red, acidine orange and 3,3-bis-(2-thienyl-4,4-difluoro-4-bora-3a,4a-diaza)-9-indacene.

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5. (original) The method of claim 4 wherein the fluorescent radical is a fluorescein derivative.
6. (previously presented) The method of claim 5 wherein the biotin selective protein is avidin or streptavidin; wherein the binding radical is biotin; and wherein the fluorescent radical is 5-([4,6-dichlorotriazin-2-yl]amino)fluorescein.
7. (original) The method of claim 1 wherein the proteases are viral proteases.
8. (previously presented) The method of claim 7 wherein the proteases are selected from the group consisting of human immunodeficiency virus proteases and herpes proteases.
9. (previously presented) The method of claim 8 wherein the herpes viruses proteases are selected from the group consisting of human cytomegalovirus proteases, mouse cytomegalovirus proteases, herpes simplex virus subtype 1 proteases and herpes simplex virus subtype 2 proteases.
10. (previously presented) The method of claim 2 wherein the substrates are selected from the group consisting of Biotin- $\gamma$ -Abu-Gly-Val-Val-Asn-Ala-Ser-Ala-Arg-Leu-Lys-5-([4,6-dichlorotriazin-2-yl]amino)fluorescein-NH<sub>2</sub> [SEQ ID NO: 3] and biotin- $\gamma$ -Abu-Ser-Gly-Asn-Tyr-Pro-Ile-Val-Gln-Lys-5-([4,6-dichlorotriazin-2-yl]amino)fluorescein-NH<sub>2</sub> [SEQ ID NO: 4].
11. (previously presented) A method for identifying compounds which inhibit a protease, said method comprising a) incubating a mixture of said protease, the compound, and a substrate having both a fluorescent radical and a radical capable of binding to an anchor; b) binding the substrate to the anchor; c) measuring the fluorescence polarization of the emitted light; and d) calculating the amount of protease inhibition.

Claims 12-15 (canceled)

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16. (previously presented) A method for determining the activity of a protease, said method comprising

- a) incubating a mixture of said protease and a substrate selected from the group consisting of biotin- $\gamma$ -Abu-Gly-Val-Val Asn-Ala-Ser-Ala-Arg-Leu-Lys-5-([4,6-dichlorotriazin-2-yl]amino)fluorescein NH<sub>2</sub> [SEQ ID NO: 3] and biotin- $\gamma$ -Abu-Ser-Gly-Asn-Tyr-Pro-Ile-Val-Gln-Lys-5-([4,6-dichlorotriazin-2-yl]amino)fluorescein-NH<sub>2</sub> [SEQ ID NO: 4];
- b) binding the substrate to an anchor comprising a biotin selective protein;
- c) measuring the fluorescence polarization of the mixture; and
- d) correlating the measured fluorescence polarization to protease activity.